Reference: *Biol. Bull.* **166**: 368–383. (April, 1984)

ENTOCLADIA ENDOZOICA SP. NOV., A PATHOGENIC CHLOROPHYTE: STRUCTURE, LIFE HISTORY, PHYSIOLOGY, AND EFFECT ON ITS CORAL HOST

W. M. GOLDBERG, J. C. MAKEMSON, AND S. B. COLLEY

Department of Biological Sciences, Florida International University, Miami, Florida 33199

Abstract

A new species of the marine microalgal genus *Entocladia* (Ulvellacea, Chlorophyta) is described from cultured material and from its natural habitat, the skeleton of a gorgonian coral (*Pseudoplexaura* spp.). Host tissues react to the presence of this filamentous chlorophyte by producing a capsule composed primarily of scleroprotein skeleton, and secondarily of calcareous spicules. The skeletal capsule separates the algae from contact with host tissue but in doing so the skeleton loses more than 60% of its tensile strength and over 90% of its elasticity. The coral colony readily breaks apart at the site of the weakened skeleton, exposing the contained algal filaments to relatively high light fields and sea water, an amino acid-depleted environment. These conditions lead to rapid cytological changes that convert the alga to a reproductive state. Regeneration of damaged host tissue re-seals the capsule and causes resumption of the vegetative condition typical of enclosed algae. Experiments with native and cultured material suggest that host-derived amino acids, especially tyrosine or cystine, play a key role in regulating the reproductive condition of *Entocladia* filaments.

Introduction

Algal pathogens have been associated with a wide variety of host taxa but have been noted only occasionally in the literature. A form of lymphadenitis in cattle and sheep is apparently caused by a green alga (Rogers *et al.*, 1980) while other chlorophytes attack higher plants (Joubert and Rijkenberg, 1971) or cause pathogenesis in certain bivalve molluscs (Naidu, 1971). Recently, Morse *et al.* (1977, 1981) described the occurrence of tumor-like growths associated with a siphonaceous green alga in sea fans of the genus *Gorgonia*. A different chlorophyte is associated with abnormal growths in gorgonian corals of the genus *Pseudoplexaura* particularly in the shallows of the Florida Keys (Fig. 1). Of 1377 colonies examined from the northern Florida Keys (Soldier Key) to the Dry Tortugas, one third bear nodules. In certain reef areas containing significant *Pseudoplexaura* populations, up to 54% of the colonies exhibit this pathology (Goldberg and Makemson, 1981). We have identified the pathogen as *Entocladia endozoica* sp. nov., a marine microalga belonging to the recently resurrected family Ulvellaceae (Ulvales, Chlorophyta) (O'Kelly and Yarish, 1980; O'Kelly, 1983). In this report we describe the alga as a species that is remarkable not only for its endozoic habitat, but also for its pathogenic effect on host skeleton formation, which in turn appears to control the alga's life history. Aspects of the host's cellular response are also considered.

Received 28 October 1983; accepted 25 January 1984.

FIGURE 1. *Pseudoplexaura flagellosa* (Houttuyn) with algal nodules, depth 3 m, Bache Shoal, Florida Keys.

MATERIALS AND METHODS

Isolation and culture of the alga

Tumors were collected from several species of *Pseudoplexaura* in Florida and the Netherlands Antilles. Algal filaments were removed by forceps from surface sterilized (alcohol) tumors and washed repeatedly on Whatman filters with artificial sea water (Baumann *et al.*, 1971). Sterile technique was used in all procedures. The alga was isolated in pure culture by streaking agar plates containing Provasoli enrichments (McLachlan, 1973) added to artificial or natural sea water with the addition of 10 m*M* Tris buffer, pH 7.5. Cultures were grown at 25°C under a bank of cool white fluorescent lamps at 1000 lux on a 12:12 cycle. Within two weeks, dark green colonies (see Results, Fig. 4) became visible on the plates. The colonies were re-streaked on the same medium and on Difco-Marine Agar to check for purity and bacterial contamination. Stock cultures were maintained as slants or plates at 25°C (12:12) with bimonthly transfers. Morphological and growth studies required transfer of cells from the agar surface to a modified Provasoli's (liquid) medium, followed by gentle glass homogenization. Cell cultures (25 μl) were grown in Terasaki plates containing filter

paper wicks moistened with 2% $NaHCO_3$. Physiological experiments employed similarly prepared cell suspensions incubated in tubes at 0, 100, or 1000 lux at 25°C. Cell numbers were determined by a hemacytometer, counting only chloroplast-containing cells. Pigments were analyzed from ice cold 90% acetone extracts in a Varian 635 double beam spectrophotometer and by cellulose thin layer chromatography using a methanol:acetone:water (30:10:3) solvent system (Kleinig, 1969; O'Kelly, 1982). Pigments were compared to those of the alga *Avrainvillea nigricans* Decaisne freshly collected from Key Largo, Florida.

Radiochemicals were from New England Nuclear (Boston, Massachusetts). Scintillation counting of radioactivity employed a Beckman 9000 liquid scintillation counter using Hydromix (Yorktown Research, Hackensack, New Jersey); a final concentration of 0.1 *M* hyamine was used in samples containing labeled carbon dioxide.

Histology, ultrastructure, and skeletal mechanics

Histological observations were made only on *Pseudoplexaura flagellosa* (Houttuyn) fixed in Bouin and stained with Gomori's trichrome after normal paraffin embedment. Tissue volumes were calculated from undehydrated, fixed cross sections of branches 1 mm thick. Spicular weight was determined after treatment with commercial bleach (5% NaOCl). Material for transmission electron microscopy was fixed 2–4 hours at 4°C in several primary fixative mixtures. Most frequently we used 2% glutaraldehyde and 4% paraformaldehyde in 0.1 *M* cacodylate buffer. Addition of sucrose brought the specific gravity of the mixture to sea water tonicity. Post-fixation employed 1% OsO_4 in the same buffer and temperature for 1 hour. Long dehydration times were required for proper infiltration and embedment in Polybed 812 (Polysciences, Warrington, Pennsylvania). In later phases of this study we found that embedment with Spurr resin (Polysciences) gave better results. Silver to gold sections were cut with a diamond knife, placed on uncoated copper grids, and photographed with a Philips 200 electron microscope.

Primary fixation for scanning electron microscopy was as above. Fixed algal swarmers from cultured material were allowed to settle for 15 minutes on Nucleopore filters (0.22 μm) that had been treated with a 0.1% aqueous solution of polylysine (Sigma, MW = 350,000). This procedure firmly binds small specimens to the filter (*cf.*, Hayat, 1978). Filters and tumor material were then dehydrated in graded ethanols and critical point dried from liquid CO_2. After examination with a compound microscope, sections were cut out, mounted on studs with double stick tape, sputter coated with Au/Pd, and photographed using ISI DS130 and Super IIIA scanning electron microscopes.

Infected and uninfected skeleton (1 to 3 mm diameter) previously dried at room temperature was thoroughly rehydrated in sea water and subjected to mechanical stress in a Scott tensile stress tester using step-wise loading. Elongation of the skeleton was recorded as a function of the original length. Cross sectional area of tumorous material was taken as the mean diameter above and below the infected region.

Results

Algal morphology, ultrastructure, and pigmentation

The alga from intact tumors is typically found in masses composed of long, cylindrical filaments (Fig. 2A) composed of cells 4.5 to 6.5 μm in diameter. Cell junctions lack plasmodesmata. The cytoplasm is typically vacuolate, and while some of the vacuoles are filled with a loose fibrillar matrix (Fig. 2C), they are often free of

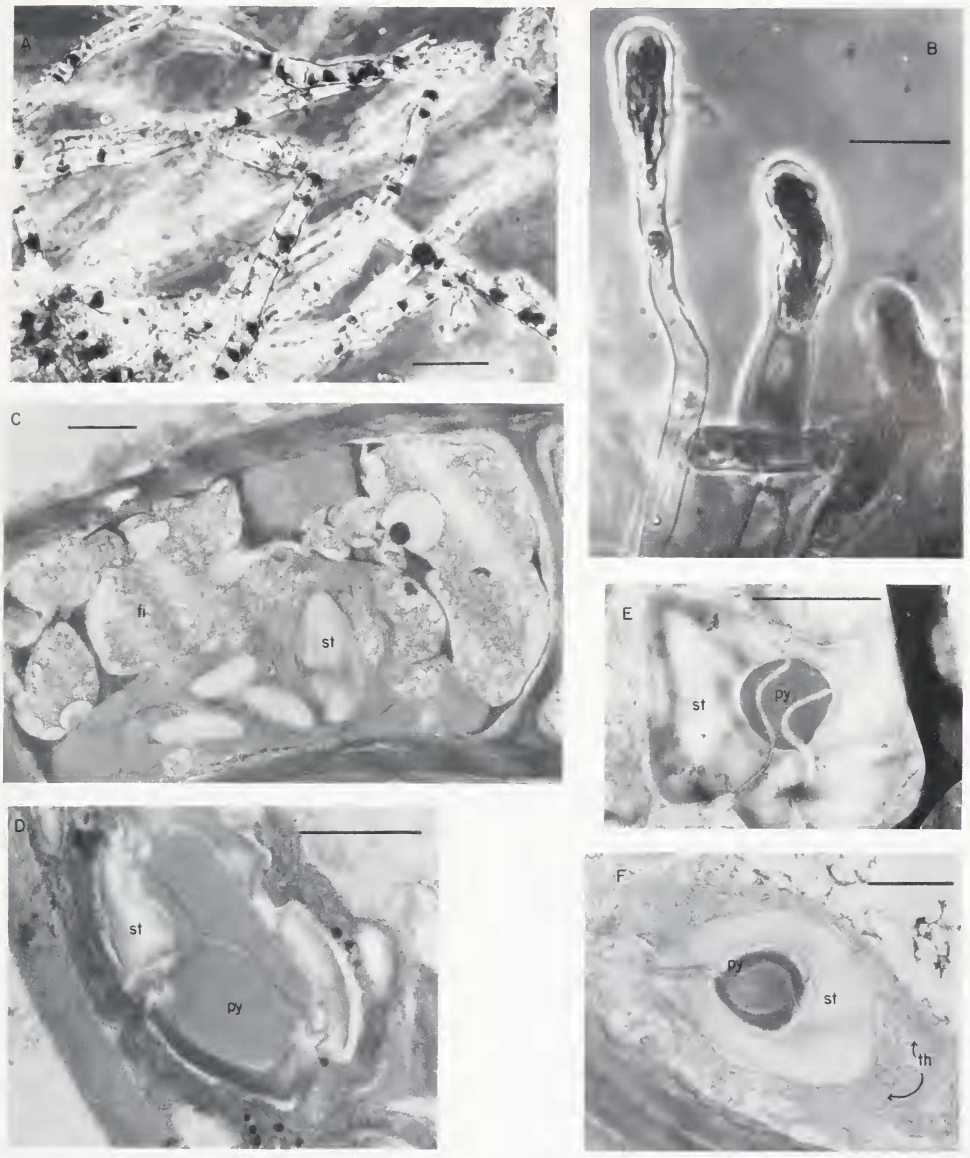

FIGURE 2. *Entocladia endozoica* field specimens. A. Vegetative filaments dissected from a nodule, scale bar is 30 μm. B. Filament ends from a nodule exposed to sea water for 10 days *in situ,* scale bar 30 μm. C. Transmission electron micrograph of a vegetative filament cell: fi = fibrilar matrix, st = starch, scale bar = 1.0 μm. D., E., and F. Pyrenoid structure: st = starch, py = pyrenoid, th = thylakoids, scale bars = 1.0 μm.

organic material. There are one or two chloroplasts which are always parietal and lack girdling lamellae. The thylakoid membranes are stacked into undulating lamellae of variable thickness. Part of the irregularity of the chloroplast is due to interruption of the lamellae by ellipsoidal starch grains (Fig. 2D, E, F) and by several pyrenoids. The pyrenoid matrix is surrounded by 2–4 starch plates and is either bisected or

trisected by chloroplast membranes. In the latter case, the membranes either may be parallel, or perpendicular and confluent (Fig. 2D, E). While some additional irregularity may be due to the vagaries of fixation, such chloroplasts are typical of the Chlorophyta (Dodge, 1973; Pickett-Heaps, 1975).

Tumors broken open and exposed to ambient sea water contain algal filaments that terminate in larger, rounded cells (Fig. 2B). These terminal cells are distinguished by enlarged and more numerous chloroplasts which usually fill and eliminate the bulk of the intracellular spaces. Within the chloroplast, the number of starch grains is increased and oil droplets, mostly 0.1–0.15 μm diameter, become conspicuous and numerous (Fig. 3). As will be described below, these rounded cells are reproductive.

Acetone extracts of whole filaments contain chlorophylls a and b as determined by spectrophotometry (Fig. 4) and thin layer chromatography (Fig. 5). The extracts also possess several carotenoids including siphonaxanthin, neoxanthin, violaxanthin, lutein, and siphonein. These were identified by comparison with pigments extracted from the siphonous alga *Avrainvillea nigricans* Decaisne. The R_f values of the carotenoids from both algae were identical and corresponded to those given by Kleinig (1969) for *A. nigricans* and other siphonous algae.

Culture studies

Pure cultures of *Entocladia* were isolated using only nonreproductive material from intact tumors. Within two weeks on Provasoli's agar, the alga produced compact colonies composed of both prostrate and erect filaments (Fig. 6). When transferred to fresh liquid culture, these cells became ellipsoid and produced numerous chloroplasts (Fig. 7A). In nitrate and phosphate enriched media following medium replenishment,

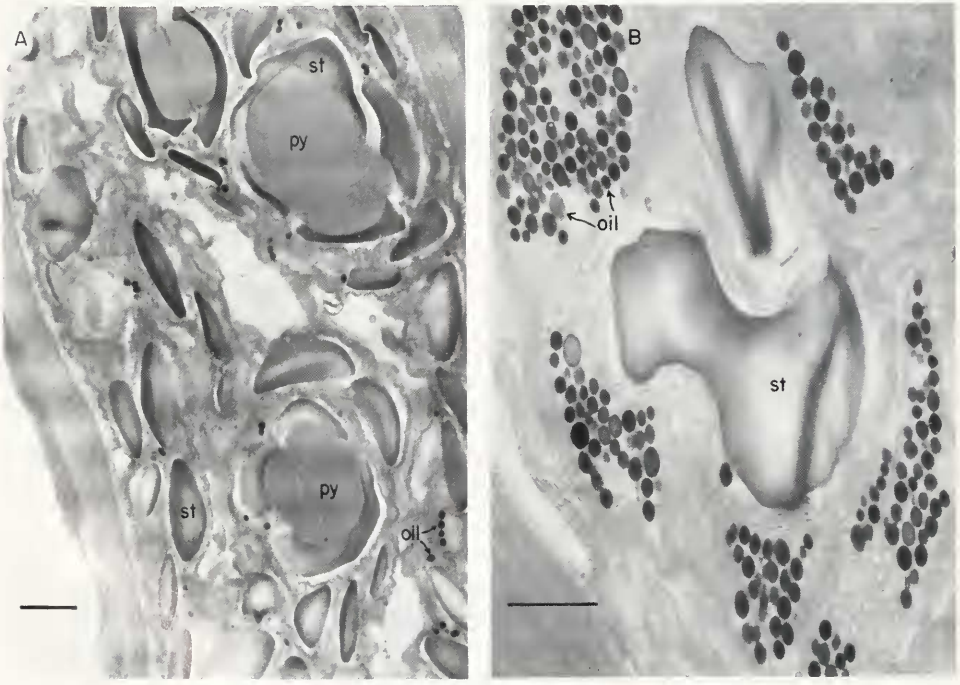

FIGURE 3. Numerous starch and oil droplets typical of reproductive cell chloroplasts, scale bars = 1.0 μm.

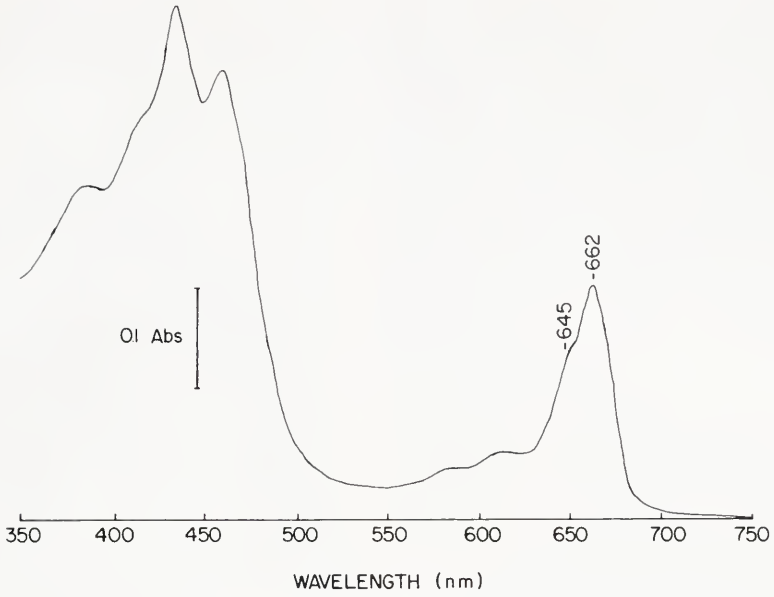

FIGURE 4. Spectrum of *E. endozoica* pigments (grown in culture) solubilized by 90% acetone.

these pre-reproductive cells enlarged and eventually became flask shaped (Fig. 7C, E) releasing swarmers. We have not determined whether these are zoospores or gametes. The swarmers were released through a single exit papilla (Fig. 7E) and possessed two flagella, but some had three and in one instance a pentaflagellated swarmer was observed (Fig. 8A, B, C).

Changes in inorganic constituents of the medium did not alter the ellipsoid morphology, although in phosphate and/or nitrate depleted media, setae were produced from bulbous extensions of the sub-polar regions (Fig. 7D). Organic carbon sources such as glucose, glycerol, acetate, glutamate, and aspartate were also ineffective. However, filaments grown in casamino acid-enriched media became elongated and produced filaments remarkably similar to those found in the tumors (Fig. 2A). The effect of casamino acids could be reproduced only by tyrosine or cystine at concentrations from 10^{-3} to 10^{-5} *M*.

Studies following the uptake and distribution of labeled substrates indicated that *Entocladia* took up carbon dioxide as well as amino acids from an amino acid mixture linearly over a one hour incubation period; glucose, glutamate and tyrosine were not taken up. After 1 hour, 89 to 96% of the HCO_3^- taken up was found in the cold TCA-insoluble fraction, and as expected, the amount of HCO_3^- taken up in the dark was only 1 to 4% of that taken up in the light (Table I). Amino acid uptake was small compared to HCO_3^-, and about 60% remained TCA soluble. Amino acid uptake was not affected by the presence of tyrosine in the growth medium and was only diminished 30–40% in the dark indicating that heterotrophy is not significant compared to the alga's autotrophic nutrition. These results have been duplicated with algal filaments isolated from freshly collected tumors (Makemson, unpub. data).

Effect on the host

The tumor-like swellings characteristic of the presence of *Entocladia* consisted primarily of the algal mass confined by laminated walls of host skeleton (Fig. 9B, C).

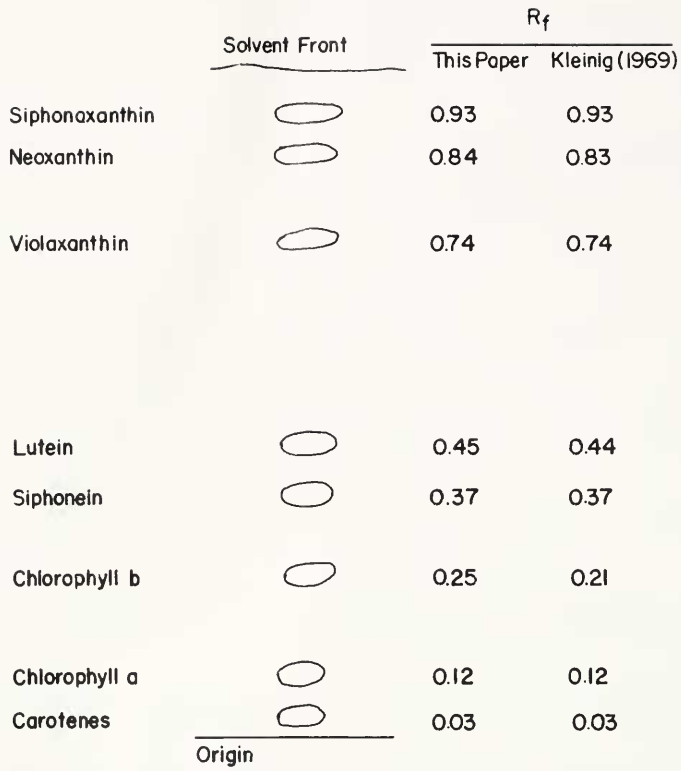

FIGURE 5. Thin layer chromatography of acetone soluble pigments from *E. endozoica* on cellulose using methanol:acetone:water (30:10:3).

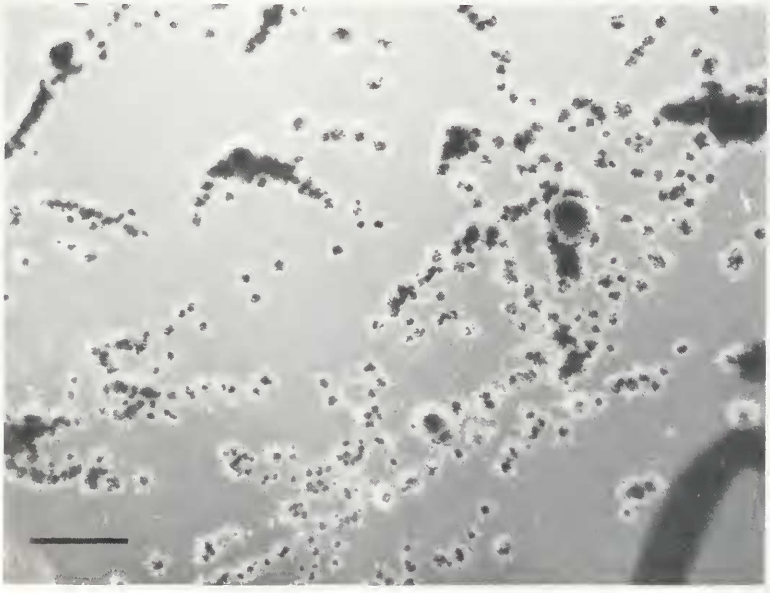

FIGURE 6. *E. endozoica* colonies on Provasoli's agar medium. Scale bar = 10 mm.

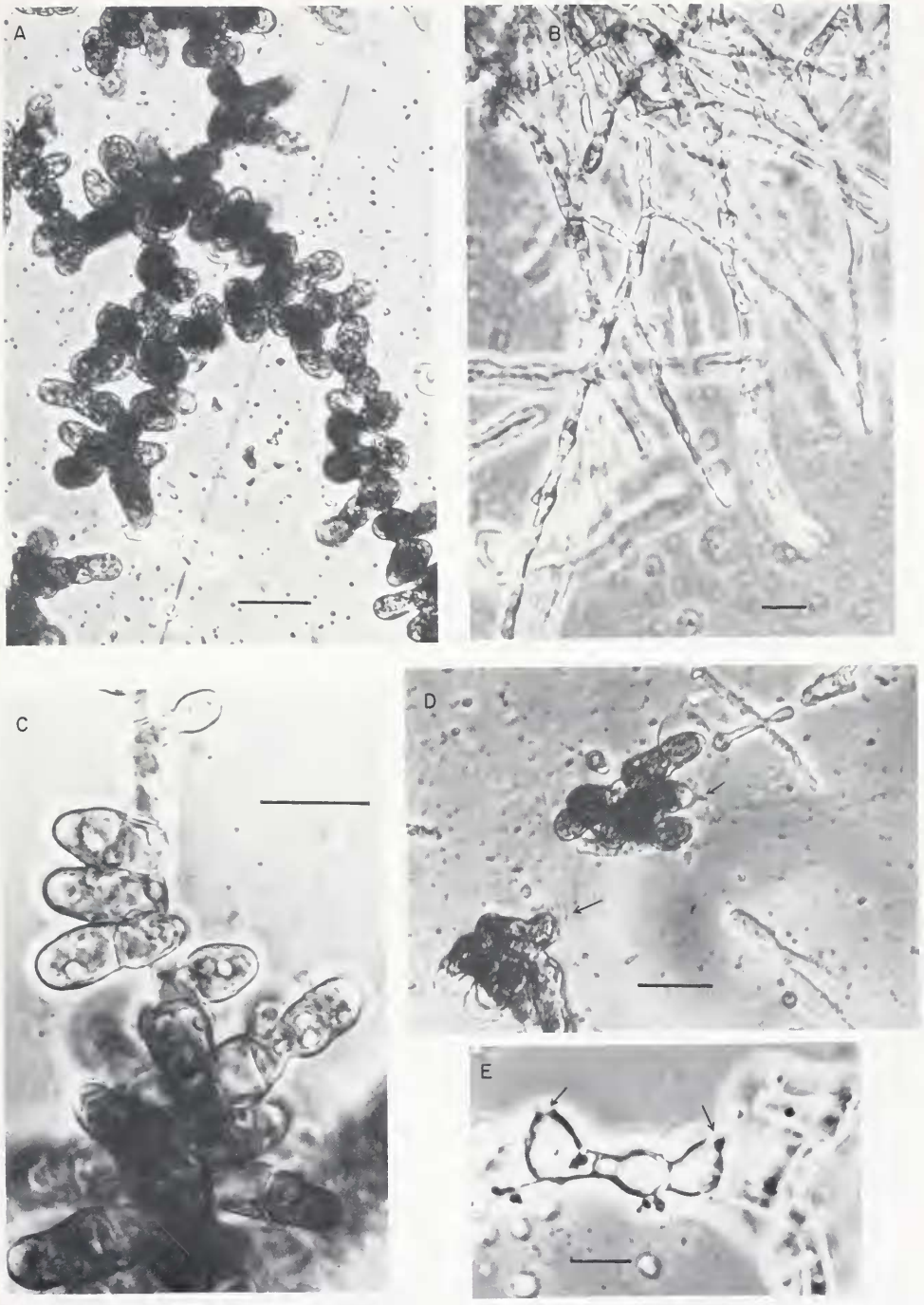

FIGURE 7. *E. endozoica* cells in pure culture. A. Pre-reproductive cells full of chloroplasts. B. Vegetative cells grown with tyrosine, 10^{-4} *M*. C. Intermediate stage in swarmer production. D. Setae originating from bulbous extensions of pre-reproductive cells (at arrows). E. Empty sporangia (or gametangia) with single exit papillae (at arrows). Scale bars = 30 μm.

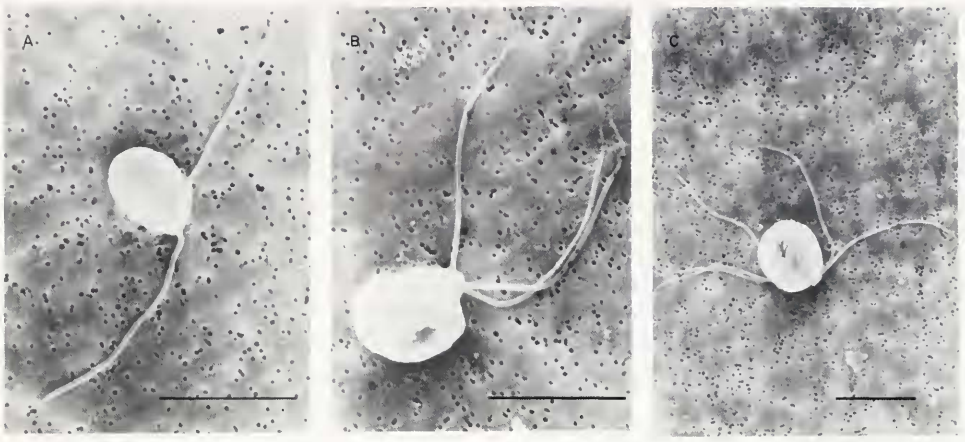

FIGURE 8. Scanning electron micrographs of swarmers possessing 2 (A), 3 (B), and 5 (C) flagella. Scale bars = 5 μm.

Much of the tumor histology is explainable simply by the mechanical strain of the algal mass and the increased surface area of host tissue that results from it. There was no pathology associated with the polyp layer surrounding the tumors. A study of reproduction in six normal and six infected colonies, for example, showed no differences in either the maturation or the development of gonadal material (Goldberg, unpub. data).

The major histopathology of the host involved production of abnormally large numbers of scleroblast cells and granular amoebocytes. The scleroblasts proliferated in the periaxial area (axial sheath) closest to the infected skeleton. Here the spicular weight per cm^3 of host tissue was 41.2% greater than in normal hosts ($n = 11$, $P = <0.03$). The resulting mass of purple sclerites appeared to form a loose calcitic capsule around the infected skeletal region (Fig. 9A). The size of the axial sheath sclerites was within the range found in uninfected specimens (110–214 μm), however, there appeared to be a greater proportion of the ornate, mature spicules in infected colonies.

The granular amoebocyte, normally present in small numbers, accumulated in the spaces of the periskeletal mesoglea when *Entocladia* was present (Fig. 10A). This occurred whether or not the alga was totally confined by host skeleton. When algal filaments extended beyond the skeletal boundary, the amoebocytes' numerous vesicles were released by a process that appeared to involve cell lysis (Fig. 10B), coating the algae with mesoglea like (fibrous, PAS-positive) material. The coated algae (Fig. 10C) were subsequently encapsulated by skeletogenic epithelium.

Skeletal mechanics

Comparison of stress/strain relationships in infected and uninfected skeletal material is shown in Figure 11. Normal *P. flagellosa* had a sigmoidal stress curve similar to keratin, antipathin, and other moderately cross-linked fibrous proteins (Wainwright *et al.*, 1976), but with apparently greater extensibility. The mean elastic modulus in the linear range was very close to 1. With continued stress the skeletal strain increased more rapidly, to an average ultimate tensile strength of 28 kg mm^{-2} (274 MN^{-2}). Uninfected skeletal material can extend to over double its original length in the

TABLE I

Uptake of $H^{14}CO_3^-$ and ^{14}C-amino acid mixture by cultures of Entocladia *filaments*

Culture[a]	Incubation[b]	DPM taken up[c]	f-moles/cell[d]
Provasoli's Broth +$H^{14}CO_3$	light	35838 total	47
		32218 TCA ppt	42
	dark	688 total	0.9
		102 TCA ppt	0.1
+^{14}C-aa's[e]	light	9486 total	0.11
		3860 TCA ppt	0.041
	dark	7075 total	0.11
		3159 TCA ppt	0.037
Provasoli's Broth + Tyrosine + $H^{14}CO_3$	light	32684 total	105
		31723 TCA ppt	102
	dark	513 total	1.6
		80 TCA ppt	0.26
+^{14}C-aa's	light	9151 total	0.26
		2721 TCA ppt	0.08
	dark	6096 total	0.17
		1646 TCA ppt	0.047

[a] Cultures were vegetative cells grown in Provasoli's medium with and without 1 m*M* tyrosine. Cells were harvested and resuspended in broth without tyrosine; culture grown without tyrosine contained 9×10^4 cells/ml and the tyrosine-grown culture contained 4×10^4 cells/ml.

[b] Incubation of 5.0 ml of cell suspension in flasks with the appropriate label added as 20 μl. Dark incubation was in foil-wrapped flasks; both incubated at 27°C at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ using cool white fluorescent illumination for 1 hour.

[c] Radioactivity taken up was determined by filtration of 2 ml of incubation mixture onto 0.45 μm pore size filters, rinsed with three washes of artificial sea water (total) or 5% TCA (TCA ppt), and placed immediately into 5 ml of Hydromix scintillation mixture. Counts per minute were corrected for counting efficiency, background, and quench.

[d] Specific activity of each label was used to calculate amounts of material taken up and divided by the average number of cells on each filter.

[e] L-amino acid mixture, NEC-445 New England Nuclear, Boston, Massachusetts, contains 15 amino acids in the same relative proportions as found in a typical algal protein hydrolysate. 0.1 mCi/ml used undiluted with cold amino acids.

hydrated state. Skeletal material infected with algae, on the other hand, showed a mean decrease in the tensile strength of over 60% and a decrease in elasticity of over 90%.

DISCUSSION

Systematic assessment

Entocladia's thallus morphology, pigment composition, and chloroplast ultrastructure are typical of the Chlorophyta. However, its assignation to lower taxa such as the Chaetophoraceae is a matter of opinion (Bold and Wynne, 1978). Certain marine chaetophoracean genera have been placed recently in the restructured family Ulvellacea based upon their life history, chloroplast pigments and ultrastructural features (O'Kelly and Yarish, 1981; O'Kelly, 1983; O'Kelly and Floyd, 1983). The

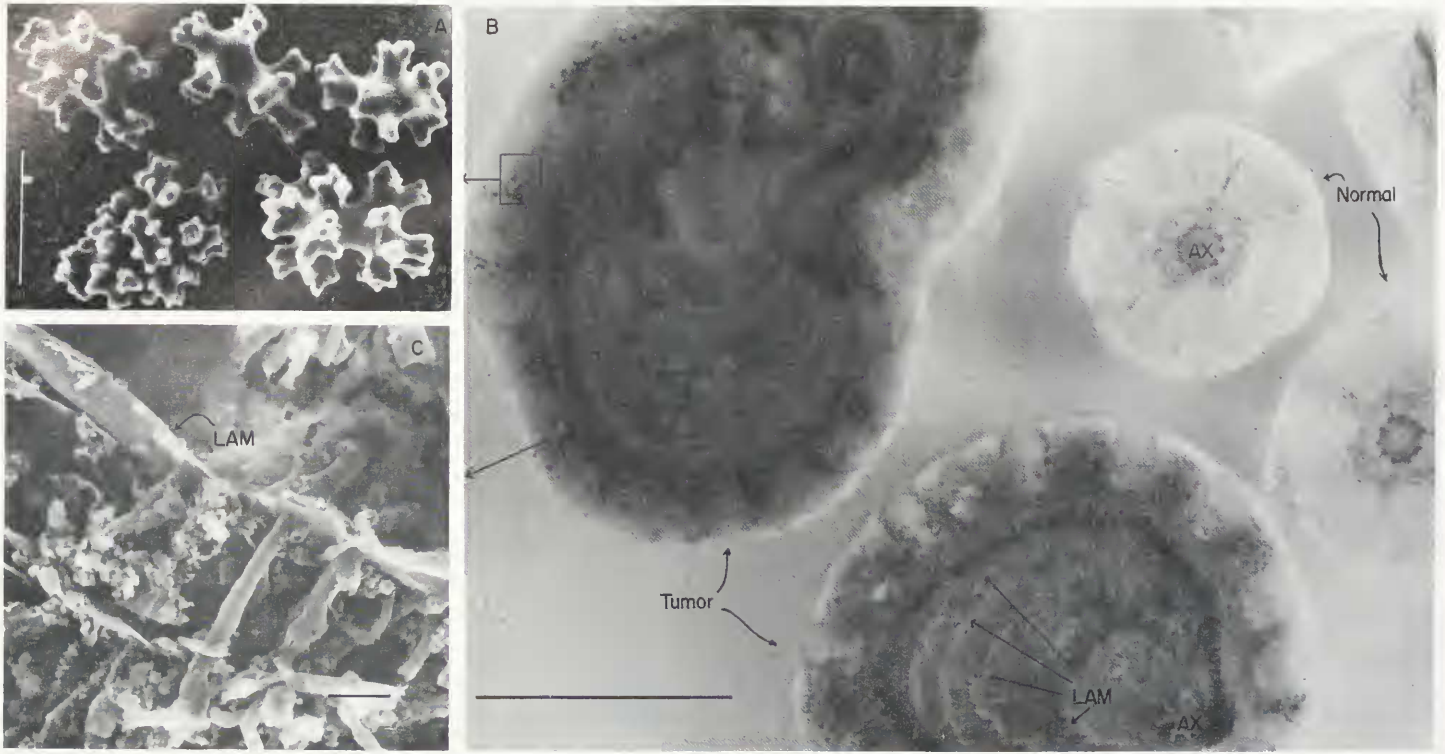

FIGURE 9. Comparison of normal and infected corals. A. Spicules are 40% more abundant in the infected condition on a weight per unit tissue volume basis. Scale bar = 100 μm. B. Cross sections comparing infected material with normal *P. flagellosa* (upper right). Laminations (lam) of axial skeleton are separated by radially oriented algal filaments. Uninfected skeleton (ax) is solid; individual laminae are fused. Scale bar = 50 μm. C. Scanning electron micrograph of algae and interposed host skeleton. Scale bar = 50 μm.

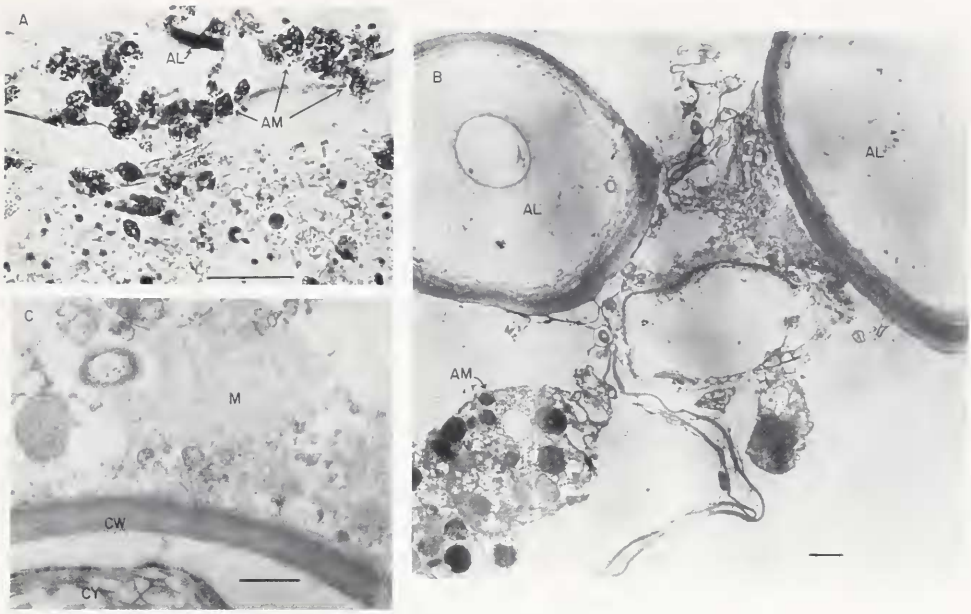

FIGURE 10. Association of coral amoebocyte and algal filaments. A. Light micrograph of amoebocytes aggregated near an algal filament; note PAS-positive vesicles; scale bar = 50 μm. B. Low power electron micrograph of amoebocyte lysing and releasing contents near two algal filaments; scale bar = 1 μm. c. Mesoglea coating an algal filament; scale bar = 1 μm. AL, algae; AM, amoebocyte; CW, cell wall; CY, chloroplast; M, mesoglea.

latter authors give familial characters that agree with our material. These include branching, filamentous, chlorophytic microalgae that may form pseudoparenchymatous masses with uninucleate cells containing parietal chloroplasts with one or more pyrenoids; colorless, nonseptate, anucleate setae may be present depending upon environmental conditions, especially nutrient depletion. Ultrastructural characteristics first described by Stewart *et al.* (1973) indicate that ulvellacean genera may also be distinguished by their lack of plasmodesmata and the presence of traversing thylakoid membranes in the pyrenoid. Our material agrees with this diagnosis (Fig. 2D, E, F) but is clearly different (segregative cell division) from the siphonaceous alga found in *Gorgonia* by Morse *et al.* (1977, 1981).

The genus *Entocladia* has been circumscribed by O'Kelly and Yarish (1981). Our material conforms to their characteristics with the exception that the number of flagella appears more variable. Occurrence of tri- and pentaflagellate forms may be an artifact of culture and preparation. *E. endozoica* probably produces bi- and quadriflagellate motile cells which are characteristic of this group (Yarish, 1975; Nielsen, 1979; O'Kelly and Yarish, 1980, 1981). The pigments of the genus *Entocladia* include chlorophylls a and b, carotenes, lutein, neoxanthin, siphonaxanthin, and violaxanthin (O'Kelly, 1982). The characters that distinguish *E. endozoica* from other *Entocladia* species are (1) amino acid-induced morphological changes; (2) its endozoic habitat; and (3) presence of siphonein in addition to the other carotenoid pigments.

Entocladia endozoica Goldberg, Makemson and Colley sp. nov.

Fila cylindrica 5–10 μm diametro, 9–18 μm longa unum chloroplastum parietalem lobatum habantia, uno vel aliquot pyrenoidibud bi- vel tri-lenticularibus ubi intra hospitem Pseudoplexauram. Fila aquam marinam exposita vel in substrata

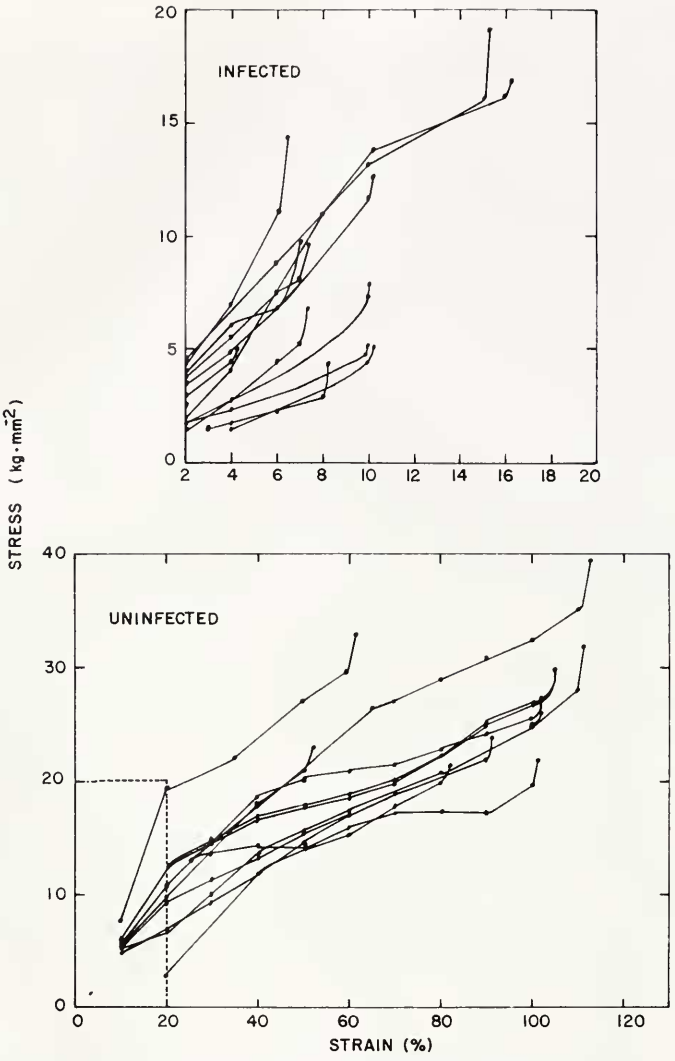

FIGURE 11. Stress-strain curves of uninfected and infected skeletons. The curves for the infected skeletons (A) fit within the dashed area of the uninfected material (B).

nutricia autotrophica sativa cellulas globosas 11.5–18 μm diametro chloroplastis multos grana multa amyli et guttulas olei signati. Sub statis phosphatis vel nitratis effoetis hae cellulae setas quoque procreare possunt. Cellulae globosae cellulas ampulliformes procreantes greges ellipsoideos 2.3–3.2 μm latos, 3.4–4.0 μm longos, 2-, 3- vel raro 5-flagellis fiunt. Cellulae reproductivae papilla singula exitus. Pigmenta chlorophylla a et b, siphonaxanthin, violaxanthin, siphonein, neoxanthin, et lutein includent. Habitat endozoica fortasse specifica generi Pseudoplexaurae.

Entocladia endozoica sp. nov.: cylindrical filaments 5–10 μm in diameter and 9–18 μm in length possessing one parietal, lobed chloroplast with one or several bi- or trilenticular pyrenoids when within its coral host. Filaments exposed to sea water or cultured in autotrophic media produce rounded cells 11.5–18 μm in diameter with

multiple chloroplasts characterized by numerous starch grains and oil droplets. Under conditions of phosphate or nitrate depletion these cells may also produce setae. Rounded cells become flask-shaped producing ellipsoid swarmers, 2.3–3.2 μm wide and 3.4–4.0 μm long with 2, 3, or rarely 5 flagella. A single exit papilla characterizes these reproductive cells. Pigments include chlorophylls a and b, siphonaxanthin, violaxanthin, siphonein, neoxanthin, and leutein. Habitat endozoic, possibly specific to gorgonian corals of the genus *Pseudoplexaura*.

Type Locality: Bache Shoals, northern Florida Keys, depth 3 m. Holotype specimens have been deposited at the National Herbarium (Smithsonian Institution, Washington, DC 20560) and pure cultures with the Culture Collection of Algae of the University of Texas (Austin, TX 78712).

Biological significance

The peculiar habitat of *E. endozoica* has in addition to systematic importance, relevance to the coelenterate host response as well as regulation of the algal life cycle. Histological evidence shows that the bulk of the abnormal swellings consist of living algal filaments enclosed by gorgonian skeletal protein. Some specimens show filaments extending into the cellular (mesogleal) areas of the host whereupon they become surrounded by granular amoebocytes. Although the precise function of these host cells is unclear, this interaction seems to be associated with encapsulation. Thus, what appear to be algal filaments penetrating the skeleton (Fig. 9C) are more likely algae in the process of being covered or surrounded by skeletal material. The fact that the algae are only occasionally able to break free from this matrix may be indicative of an efficient host response rather than an algal-induced disruption of host skeletogenesis. The proliferation of spicular material may serve as reinforcement for the capsule, preventing the algae from interacting with the polyp layer. Nonetheless, from the viewpoint of host defense mechanisms, the response is only partly competent since the alga remains viable within the host skeleton.

In the enclosed tumor, *E. endozoica* filaments are uniformly elongated. These vegetative filaments grow only in media enriched by casamino acids, cystine or tyrosine in particular, the latter without being accumulated by the cells. Since gorgonian skeletons concentrate tyrosine (Goldberg, 1976, 1978) it is possible that the skeletal milieu is tyrosine-enriched, thereby exerting a regulatory rather than a nutritional role in algal morphology and reproduction. This is supported by the miniscule uptake and incorporation of radiolabeled amino acid mixtures (Table I) and tyrosine.

Growth and proliferation of *Entocladia* filaments result in host skeletal dysplasia, with consequent reduction in both the tensile strength of the skeleton and its extensibility. Although we have not performed other measurements such as cyclic stress-strain and stress relaxation tests, the data suggest that turbulent flows or other forms of mechanical stress are likely to cause preferential breakage of the coral colony at the tumor site. Open tumors can be found in normal field conditions and always contain rounded reproductive filaments (Fig. 2). Tumors cut open artificially, exposing the vegetative filaments to sea water, produce reproductive filaments within ten days; under laboratory conditions only four days are required. Sea water as a cystine-tyrosine depleted medium appears to be an essential factor in this conversion both *in situ* and in the laboratory. The other factor is a considerable increase in light intensity. A tumor cross section 1 cm thick (with external host tissue) reduces ambient light intensity by over 99%. Assuming 120,000 lux as an average condition, (cloudless day, zenithal sun) for surface irradiance (Sverdrup *et al.*, 1942) and a 40% reduction by 3 meters of type III ocean water (Gordon and Dera, 1969), the average amount

of light penetrating a tumor should be less than 720 lux. Exposure by tumor breakage should suddenly expose algal filaments to 2 orders of magnitude more light. Experiments involving order of magnitude increases in light greatly increase growth rates (data not shown) but fail to produce reproductive cells, reinforcing our suggestion that nutritional factors, especially the lack of tyrosine or cystine, play a key role in initiating the reproductive process.

Although not observed in nature, motile cells are likely released from opened tumors before host tissue regenerates, closing over what has become an apical tumor. During the summer months, open tumors 2–3 cm in diameter heal completely within 7–10 days. Thus regeneration as well host reproduction (described above) appear to be unaffected by this association. Moreover, there is no evidence that *Pseudoplexaura* suffers from peripheral necrosis or erosion such as that described from algal-infected sea fans by Morse *et al.* (1977). Our previous study of the ecology and distribution of the tumor phenomenon (Goldberg and Makemson, 1981) showed no obvious correlation with known environmental stress areas in Florida. Furthermore, we have no evidence to indicate that the relationship described here is in the long run harmful to the host. In fact, broken *Pseudoplexaura* branches may result in vegetative propagation of the coral (*cf.,* Walker and Bull, 1983), although we have no data on branch survival. These observations suggest a close relationship between pathogen and host: *E. endozoica* is poised to respond quickly to autotrophic conditions and relatively high light fields; this exposure in turn, is made possible by the vegetative growth of the alga and the resulting loss in host skeletal strength. Further work will be necessary to determine if various pathogenic algae are genus-specific as first suggested by Morse *et al.* (1981). Such studies should provide fresh insights into the newly emerging field of coral reef pathology and open new avenues of research in alga-invertebrate symbioses.

Acknowledgments

The authors are grateful to Dr. C. J. O'Kelly for valuable criticism, to Dr. Helen Correll for the Latin description, and Ana Ferraz-Lago for her assistance with thin-layer chromatography. Portions of this work were supported by NSF Grant PCM 79-20522.

LITERATURE CITED

Baumann, P., L. Baumann, and M. Mandel. 1971. Taxonomy of marine bacteria: the genus *Beneckea*. *J. Bacteriol.* **107**: 269–294.

Bold, H. C., and M. J. Wynne. 1978. *Introduction to the Algae: Structure and Reproduction.* Prentice-Hall, Englewood Cliffs, New Jersey. 706 pp.

Dodge, J. D. 1973. *The Fine Structure of Algal Cells.* Academic Press, New York. 261 pp.

Goldberg, W. M. 1976. A comparative study of the chemistry and structure of gorgonian and antipatharian coral skeletons. *Mar. Biol.* **35**: 253–267.

Goldberg, W. M. 1978. Chemical changes accompanying maturation of the connective tissue skeletons of gorgonian and antipatharian corals. *Mar. Biol.* **49**: 208–210.

Goldberg, W. M., and J. C. Makemson. 1981. Description of a tumorous condition in a gorgonian coral associated with a filamentous green alga. *Proc. 4th Int. Coral Reef Symp.* **2**: 685–697.

Gordon, H. R., and J. Dera. 1969. Irradiance attenuation measurements in seawater off southeast Florida. *Bull. Mar. Sci.* **19**: 279–285.

Hayat, M. A. 1978. *Introduction to Biological Scanning Electron Microscopy.* University Park Press, Baltimore, Maryland. 323 pp.

Joubert, J., and F. Rijkenberg. 1971. Parasitic green algae. *Ann. Rev. Phytopathol.* **9**: 45–64.

Kleinig, H. 1969. Carotenoids of siphonous green algae: A chemotaxonomic study. *J. Phycol.* **5**: 281–284.

McLachlan, J. 1973. Growth media—marine. Pp. 25–52 in *Handbook of Phycological Methods,* J. Stein, ed. Cambridge Univ. Press, Cambridge.

MORSE, D. E., A. MORSE, AND H. DUNCAN. 1977. Algal "tumors" in the Caribbean sea fan, *Gorgonia ventalina. Proc. 3rd Int. Coral Reef Symp.* **1:** 623–629.

MORSE, D. E., A. MORSE, H. DUNCAN, AND R. K. TRENCH. 1981. Algal tumors in the Caribbean octocorallian, *Gorgonia ventalina:* II. Biochemical characterization of the algae, and first epidemiological observations. *Bull. Mar. Sci.* **31:** 399–409.

NAIDU, K. S. 1971. Infection of the giant scallop *Placopecten magellanicus* from Newfoundland with an endozoic alga. *J. Invert. Pathol.* **17:** 145–157.

NIELSEN, R. 1979. Culture studies on the type species of *Acrochaete, Bolbocoleon,* and *Entocladia* (Chaetophorales, Chlorophyceae). *Bot. Not.* **132:** 441–449.

O'KELLY, C. J. 1982. Chloroplast pigments in selected marine Chaetophoraceae and Chaetosiphonaceae (Chlorophyta): The occurrence and significance of siphonaxanthin. *Bot. Mar.* **25:** 133–137.

O'KELLY, C. J. 1983. Observations on marine Chaetophoraceae (Chlorophyta). IV. The structure, reproduction, and life history of *Acrochaete geniculata* (Gardner) comb. nov. *Phycologia* **22:** 13–22.

O'KELLY, C. J., AND C. YARISH. 1980. Observations on marine Chaetophoraceae (Chlorophyta). I. Sporangial ontogeny in the type species of *Entocladia* and *Phaeophila. J. Phycol.* **16:** 549–558.

O'KELLY, C. J., AND C. YARISH. 1981. Observations on marine Chaetophoraceae (Chlorophyta). II. On the circumscription of the genus *Entocladia* Reinke. *Phycologia* **20:** 32–45.

O'KELLY, C. J., AND G. L. FLOYD. 1983. The flagellar apparatus of *Entocladia viridis* motile cells, and the taxonomic position of the resurrected family Ulvellaceae (Ulvales, Chlorophyta). *J. Phycol.* **19:** 153–164.

PICKETT-HEAPS, J. 1975. *Green Algae: Structure, Reproduction and Evolution in Selected Genera.* Sinauer Associates, Inc., Sunderland, Massachusetts. 606 pp.

ROGERS, R. J., M. D. CONNOLE, J. NORTON, A. THOMAS, P. W. LADDS, AND J. DICKSON. 1980. Lymphadenitis of cattle due to infection with green algae. *J. Comp. Pathol.* **90:** 1–9.

STEWART, K. D., K. R. MATTOX, AND G. L. FLOYD. 1973. Mitosis, cytokinesis, and distribution of plasmodesmata and other cytological characteristics in the Ulotrichales, Ulvales, and Chaetophorales-phylogenetic and taxonomic considerations. *J. Phycol.* **9:** 128–141.

SVERDRUP, H. U., M. W. JOHNSON, AND R. H. FLEMMING. 1942. *The Oceans their Physics, Chemistry, and General Biology.* Prentice-Hall Publ., Englewood Cliffs, New Jersey. 1087 pp.

WAINWRIGHT, S. A., W. D. BIGGS, J. D. CURRY, AND J. M. GOSLINE 1976. *Mechanical Design in Organisms.* Edward Arnold Publ. Ltd., London. 423 pp.

WALKER, T. A., AND G. D. BULL. 1983. A newly discovered method of reproduction in gorgonian coral. *Mar. Ecol. Prog. Ser.* **12:** 137–143.

YARISH, C. 1975. A cultural assessment of the taxonomic criteria of selected marine Chaetophoraceae (Chlorophyta). *Nova Hedwigia* **26:** 385–430.